

## An improved method of diet preparation for toxicological feeding experiments

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### Summary

Groups of 30 rats each were fed 1 of 4 diets to determine feeding preference for freeze-dried meal compared to dry meal, and to determine if 3% agar is a desirable component of freeze-dried meal. The diets were: dry meal, meal+distilled water(freeze-dried), meal+3% agar in distilled water(freeze-dried), and meal+3% agar in distilled water(gel). Diet preparation, and the method for introducing a toxin in a toxicological study, is described. Feed consumption, bodyweight, and observational data were collected. Rats ate freeze-dried meal in amounts comparable to dry meal. There were no significant variations in feed conversion ratios among the dietary treatment groups. Agar is not required for freeze-dried diets, and we recommend that it be omitted to save resources and preparation time. Freeze-dried diets have a longer storage and cage life, thus the frequency of diet preparation, animal feeding and clean-up are reduced. For studies involving a toxin, these factors would reduce the potential for accidental exposure of personnel to potentially harmful materials.

In the design of a toxicological study, the route of exposure is a prime consideration. Potential toxins occurring in agricultural products that will be eaten should be given orally to the test species. This may be by gastric intubation or by administering the agents in feed or water. The first method is useful for experiments that utilize a relatively small number of animals over a short period of time. However, it is often preferable to include the test material in the diet. If a substance is sufficiently water soluble for the highest dose to be administered *via* the drinking water, this is perhaps the least complicated method. Other test materials that are not as soluble may have to be mixed in the feed, with the inherent problems of dust formation (Sansone, Losikoff &

Pendleton, 1977). Generation of dust during the feed mixing procedure is of concern because of danger to workers or contamination of a large area.

Methods have been developed to minimize dust generated from mixing operations. For example, aflatoxin B<sub>1</sub> was dissolved in acetone and added to an agar-based synthetic diet (Wogan & Newberne, 1967; Wogan, Paglialunga & Newberne, 1974). After thorough mixing, during which the solvent evaporated, the preparation was poured into pans and allowed to cool. According to these authors, the advantage of this gelled preparation is minimal spillage and wastage by animals and an almost complete elimination of airborne dust particles.

The method described here involves wetting rodent meal with water to reduce dust formation. A water-soluble solvent containing a toxin is then added, mixed thoroughly, and the mixture poured into trays and freeze-dried. We have successfully used this method in several recent studies (Morrissey, 1983). For the experiments described below we used solvent without any toxin. We found that freeze-dried diets, both with and without agar, were both consumed as well by rats as the dry meal.

### Materials and methods

#### *Animals*

A total of 120 12 week-old female Fischer 344 rats (F344/NHSD BR) were obtained from Harlan Sprague Dawley (Indianapolis, Indiana, USA) and acclimatized for 2 weeks. Rodent meal (Charles River Certified Rodent Meal, RMH 3200, Agway, Syracuse, NY, USA) and water were available *ad libitum* during this period. Animals were randomly assigned to 1 of 4 treatment groups (Table 1) and housed 3 to a cage in suspended wire bottom cages with automatic watering and flush systems. Racks were changed and cleaned weekly. All animals were weighed on the first and last days of a 1-week feeding period. Feed consumption was measured twice in the 1-week feeding period, except for those animals consuming diet 4, in which it was measured daily for 3 days. All animals were observed a minimum of twice per day. Temperature was maintained at  $22 \pm 2^\circ\text{C}$  and relative humidity at

\* Reference to a company or product name does not imply approval or recommendation of the product by the US department of Agriculture to the exclusion of others that may be suitable.

Table 1. Rat diets

Diet	Components	Meal(kg): water or 3% agar(L)	Freeze- dried?	No. of rats
1	meal	—	no	30
2	meal, water	1:1:1	yes	30
3	meal, 3% agar	1:1:1	yes	30
4	meal, 3% agar	1:1:1	no	30

50–70%. Fluorescent lighting was turned on at 6:00 am and off at 6:00 pm to provide a 12-h light–dark cycle.

#### Diet preparation

Fresh Rodent Meal was used for all diets. Diet 1 came directly from the supplier without any additional treatment. Diets 2–4 were prepared by weighing an appropriate quantity of feed and adding it to a large stainless steel mixing bowl on a Reynolds mixer (Maywood, Illinois, USA). For each kg of feed, 1.1 l of distilled water was heated to boiling on a hot plate, cooled to 50°C and poured into the mixing bowl with the meal. To prepare diets 3 and 4, 3% agar (Difco Laboratories, Detroit, Michigan, USA) (w/v) was added to the distilled water prior to heating. The meal and water (without agar, diet 2; with agar, diets 3 and 4) were stirred at slow speed for 30 min. Then diet 4 was sealed in labelled plastic bags, and frozen. Diets 2 and 3 were placed in labelled stainless steel freeze dry pans (28 cm × 84 cm) to a depth of about 2.5 cm. These pans were placed on the floor of a walk-in freezer to hasten the freezing process. A stainless steel cutter (28 cm × 28 cm) with blades spaced 2.5 cm apart, was used 30 min later to cut the meal into 2.5-cm (1-in) squares. Cutting must take place after the diet has hardened but prior to complete freezing. After another 2 h in the freezer, the pans were placed in a freeze-dryer (Virtis, Gardiner, NY). Minimal heat (23°C) was used during the drying process. The diets were removed after 2 days and placed in large plastic bags for storage under refrigeration. The diets were broken into cubes at this time. Prior to using diets 2–4 it was necessary to equilibrate the plastic bags containing the diets to room temperature in order to prevent water condensation on the feed. The water content of each diet was determined by weighing several samples prior to and after drying at 95°C for a 3-day period.

#### Statistics

1-way analysis of variance was used to determine significant differences ( $P < 0.05$ ) among treatment groups. Duncan's multiple range test was used for determining mean separation in cases where there was significant variation among sample means.

## Results

#### Animal observations

No animal exhibited any signs of illness during the course of this study. We noticed that several cages of animals in the diet 4 group appeared to be consuming extraordinarily large amounts of feed. By turning off the flush cycle for a few hours and observing these animals more closely, we determined that these apparent large feed consumptions were due to spillage. A small mound of diet collected under these cages, but not under the cages of any other treatment group.

#### Feed consumption/weight gain

There were no significant differences among groups with respect to initial or final body weights (Table 2). Calculated on the basis of dry feed consumed/rat/day, treatment group 4 consumed significantly more feed than the other 3 groups. This relationship held when the dry feed consumed/g of bodyweight was calculated. The average weight gain/rat/day was significantly greater in the groups consuming 3% agar (diets 3,4) compared to the group consuming dry meal. However, there were no significant differences among groups when the feed conversion (weight gained/g of dry feed consumed) was calculated.

#### Discussion

This experiment shows that rats consume freeze-dried diets in amounts comparable to dry meal. The apparent high feed consumption of diet 4 rats may be partially explained by wastage. Their weight gains were not significantly different from those animals consuming freeze-dried diets (groups 2,3), but they were almost twice the gains made by rats eating dry meal. Feed conversion ratios were similar in all groups.

Since diets 2 and 3 were consumed equally well, there appears to be no advantage to inclusion of agar in the preparation of freeze-dried diets. Agar has been used to improve the consistency of wet diets (Wogan & Newberne, 1967; Wogan *et al.*, 1974) but it is not necessary if the diet is freeze-dried. Elimination of agar and feeding a freeze-dried diet saves time and resources. Clapp and Bradbrook (1982) estimate that the cost of an agar diet study is more than 15% greater than a conventional one, due principally to increased

**Table 2.** Distribution of weight gains and feed consumptions of Fischer rats consuming diets 1-4

	Treatment group			
	1	2	3	4
Mean initial rat wt. (g $\pm$ SD)	167.1 $\pm$ 6.0	163.9 $\pm$ 6.4	165.3 $\pm$ 8.6	164.2 $\pm$ 6.4
Mean final rat wt. (g $\pm$ SD)	170.0 $\pm$ 6.6	168.6 $\pm$ 7.0	169.9 $\pm$ 9.5	166.6 $\pm$ 6.2
Dry feed/rat/day (g $\pm$ SD)	11.3 $\pm$ 0.8*	13.6 $\pm$ 1.5*	12.4 $\pm$ 1.0*	18.5 $\pm$ 5.9 <sup>i</sup>
Dry feed/average wt. of rat ( $\pm$ SD) ( $\times 10^{-2}$ )	6.7 $\pm$ 0.4*	8.2 $\pm$ 0.8*	7.4 $\pm$ 0.7*	11.1 $\pm$ 3.4 <sup>i</sup>
Average wt. gain/rat/day (g $\pm$ SD) ( $\times 10^{-1}$ )	1.4 $\pm$ 1.0*	2.2 $\pm$ 0.9* <sup>†</sup>	2.4 $\pm$ 1.4 <sup>i</sup>	2.7 $\pm$ 1.0 <sup>i</sup>
Feed conversion <sup>‡</sup> ( $\pm$ SD) ( $\times 10^{-2}$ )	1.2 $\pm$ 0.8	1.6 $\pm$ 0.6	1.9 $\pm$ 1.0	1.7 $\pm$ 0.9

\*<sup>i</sup>Values with different superscripts differ significantly ( $P < 0.05$ )

<sup>‡</sup>Feed conversion = Average weight gain/rat/day/Dry feed consumed/rat/day

labour costs for diet preparation and daily animal feeding.

Freeze drying offers other advantages over both dry and gelled diets. Dust generation should be the same as in the preparation of 3% wet agar diets, since all steps are identical through the mixing procedure. By incorporating a fluorescent indicator into a gel-based or meal diet, it was shown that a gelled diet produces less contamination than one mixed dry (Sansone *et al.*, 1977; Sansone & Fox, 1977). Feeding a dry diet is likely to increase respirable dust particles in an animal room (Clapp & Bradbrook, 1982). However, other properties of freeze-dried diets may offset this disadvantage compared to veterinary diets although these have not been studied. For example, the storage life of freeze-dried diets exceeds that of the wet diets. If a toxin is stable, it could be incorporated into a large batch of freeze-dried diet prior to initiation of the study, thus eliminating the necessity for weekly diet preparations. Wet diets should be changed daily to reduce the potential for fungal and bacterial growth but freeze-dried diets can remain in animal cages for a longer period of time without spoiling, reducing the number of times diet must be handled and total

time spent in the animal room. It is easier to clean feed hoppers that have held freeze-dried diet, and they do not require as frequent cleaning as with wet diets. We have successfully used the method reported here in studies of 6 weeks (Morrissey, 1983) and longer (Morrissey, unpublished) duration, and anticipate that it would be useful in long-term studies because of its simplicity, but we have not yet investigated this possibility.

We did not observe any group-related differences in final bodyweight or feed conversion between those rats eating wet agar-based and dry meal diets. Clapp & Bradbrook (1982) noted that those rats eating agar-based diets for their lifetime had poorer food utilization and a 25% reduction in mature bodyweight, although they were in good health. The short-term nature of the present study and the fact that animals were 12 weeks old when started on the diets may have contributed to the fact that we did not observe these effects.

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## Eine verbesserte Methode der Diätzubereitung für toxikologische Fütterungsexperimente

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### Zusammenfassung

An Gruppen von 30 Ratten wurden je eine von 4 Diäten verfüttert um Futterpräferenz für gefriergetrocknetes Futtermehl im Vergleich zu Trockenmehl zu bestimmen und um festzustellen, ob ein 3%iger Agarzusatz zu gefriergetrocknetem Futtermehl wünschenswert ist. Die Diäten waren: Trockenmehl, Mehl + destilliertes Wasser (gefriergetrocknet), Mehl + 3% Agar in destilliertem Wasser (Gel). Die Diätzubereitung und die Methode zur Beimengung eines Toxins in einer toxikologischen Untersuchung werden beschrieben. Futterverbrauch, Körpergewicht und Beobachtungsdaten wurden gesammelt. Die Ratten fraßen gefriergetrocknetes Mehl in vergleichbaren Mengen wie

Trockenmehl. Es gab keine signifikanten Abweichungen beim Futteraufwandsindex zwischen den verschieden behandelten Diätgruppen. Also wird für die gefriergetrocknete Diät nicht benötigt. Wir empfehlen, den Agarzusatz wegzulassen um Material und Zubereitungszeit einzusparen. Gefriergetrocknete Diäten haben eine längere Haltbarkeit bei der Lagerung und im Käfig. Daher wurde die Häufigkeit der Diätzubereitung, des Fütterns und des Saubermachens reduziert. Für Untersuchungen mit Toxinen helfen diese Fakten die Gefahr der versehentlichen Exposition des Personals gegenüber möglicherweise gefährlichen Substanzen zu vermindern. (G)